Stern-Volmer Quenching of Conjugated Polymers:

A Study of Fluorophore Concentration

An Undergraduate Thesis by
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Abstract

The purpose of this thesis is to re-examine the independence of Stern Volmer constants on the fluorophore concentration. Conventional theory suggests that Stern-Volmer constants are independent of fluorophore concentration. Using PPEs as fluorophore, the Stern-Volmer constants were obtained for different PPE concentrations quenched with $TbCl_3$. Stern-Volmer constants increased with decreasing PPE concentration.

Keywords:

Stern-Volmer quenching

Conjugated polymers

poly(*para*-phenylenethynylenes)

Introduction

The objective of this thesis is to investigate the dependence of fluorophore concentration with regard to Stern-Volmer quenching of conjugated polymers. This research will provide new insight on the quenching that takes place in conjugated polymers. Conjugated polymers are important as sensing materials for heavy metal ions in water, organic vapors, and explosives through fluorescence quenching via Stern-Volmer behavior.[1] The classical sensing methods are atomic absorption spectrometry, anodic stripping voltametry and X-ray fluorescence spectrometry, which all are expensive methods of sensory determination. Fluorescence quenching of conjugated polymers in the presence of analyte may provide quick inexpensive methods for the sensing of dangerous substances.

Fluorescence quenching involves two chemical species, the fluorophore and the quencher. A fluorophore is a chemical species that, when excited, emits fluorescence, and a quencher is a chemical species that reduces the fluorescence of a fluorophore. Fluorescence quenching is measured quantitatively with the Stern-Volmer equation. In **equation 1**, the quencher concentration is [Q], the Stern-Volmer constant is Ksv, Fo is the measured fluorescence intensity without quencher present, and $F_{[Q]}$ is the measured fluorescence intensity with [Q] present. After plotting (F_o / $F_{[Q]}$) against [Q], the slope can be determined to give the value of Ksv, the Stern-Volmer constant. Generally, a more sensitive system will have a steeper slope and, as a result, a higher Ksv value. The Stern-Volmer equation does not contain a variable for the fluorophore concentration and suggests that fluorescence quenching is independent of the fluorophore concentration.[2]

$$F_o/F_{[Q]} = 1 + K_{sv}[Q] \text{ or } K_{sv} = ((F_o/F_{[Q]}) - 1)/[Q]$$

Equation 1: The Stern-Volmer equation does not have a variable for fluorophore concentration, suggesting fluorophore independence

Fluorescence quenching typically occurs through the mechanisms of either static or dynamic quenching. In dynamic quenching, a collision between the quencher and fluorophore takes place during the excitation lifetime of the fluorophore. The efficiency of dynamic quenching is limited by the lifetime of the excited state of the fluorophore and the quencher concentration. In static quenching, complexation occurs in the ground state between the quenching species and the fluorophore. In this mechanism, quenching of the excited fluorophore by the complex quencher occurs upon irradiation. Due to the short lifetimes (300 ps) of PPEs, they only exhibit static quenching.

Poly(aryleneethynylene)s (PAEs) are a category of conjugated polymers by which arene groups are bonded together by alkyne linkers. Generally, PAEs present distinct chromogenic and fluorescent behavior. Though PAEs have similar structural characteristic to other conjugated systems, PAEs exhibit exceptional fluorescence capabilities when compared to other conjugated systems. Poly(*para*-phenylenethynylene)s (PPE) fall within this class of polymers, and demonstrate extraordinary fluorescence in solution and in the solid state. [1]

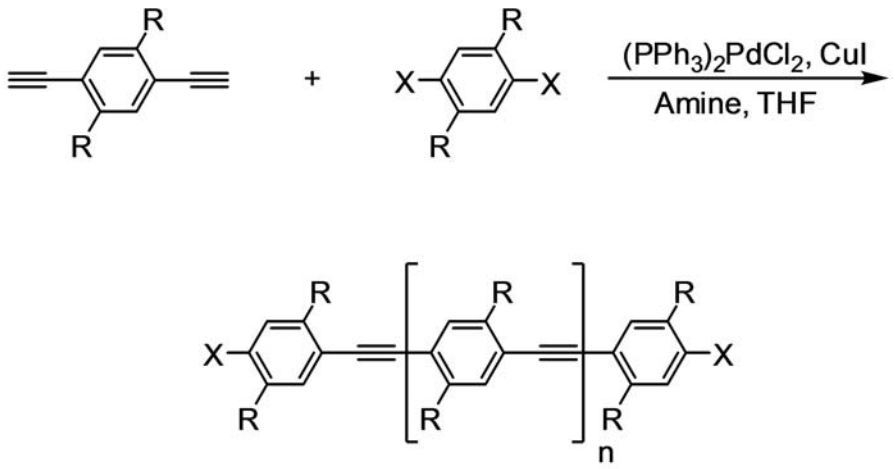
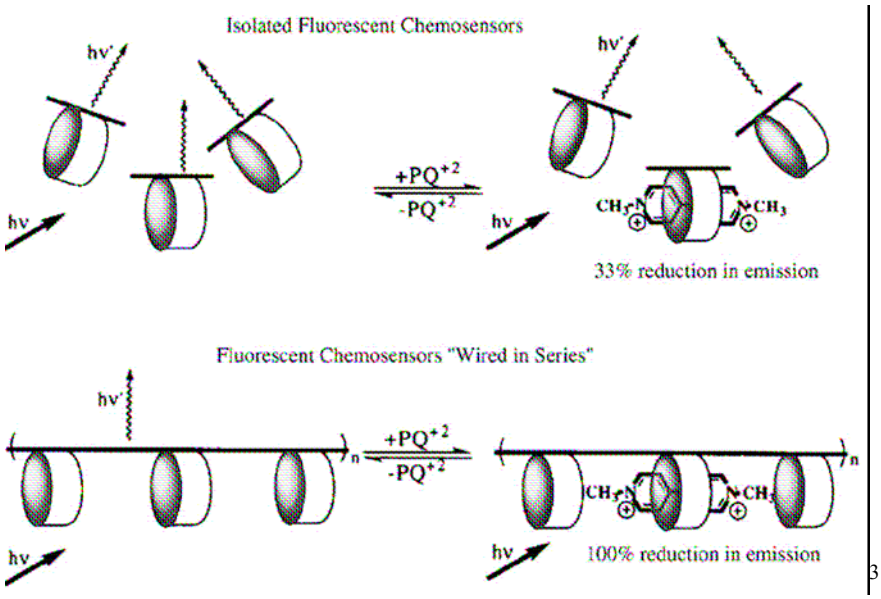


Figure 1: PPEs are conjugated molecules where the arene groups are in conjugation with the alkyne linkers. Diverse functionality can be introduced to PPEs by simply altering the R groups. n is the number of subunits.

Many quenching studies involving PPEs as a fluorophore have been conducted to evaluate the substituent effects, fluorophore arrangement, and the solvent effects on Stern-Volmer quenching. These studies have been vital in the advancements of conjugated polymeric fluorescence quenching because they have given insight on creating ideal chemo sensors. The investigation of fluorophore concentration effects on Stern-Volmer quenching behavior is at the forefront of current research, and there have not been any publications on these effects.

Research groups have placed a great emphasis of the methods in which PPE experience fluorescence quenching. There have been significant strides in the research of the molecular wire effect with regard to fluorescence quenching. The main premise is that chemical sensors attached together on a molecular backbone may act similarly to an electrical circuit wired in series. Swager demonstrated increased sensitivity to conjugated polymers when comparing quenching of PPE wired in series with PPE end-capped with anthracence units.[3] While using methyl viologen as a quencher, results showed a 67-fold increase when comparing the PPE wired in series to a non-wired PPE.

The conclusion from Swager's work is that augmented quenching occurs when the fluorophore is in a molecular wire arrangement. Like an electrical circuit, the rest of the molecular chain ceases to fluoresce when a quencher complexes to one part of the chain. As **figure 2** demonstrates, isolated chemo sensors can continue to emit fluorescence after one sensor has been quenched with quencher where as fluorescent sensors wired in series cannot fluoresce after one sensor has been quenched. Swager's work shows that fluorescent chemosensory systems wired in series are advantageous because of the increased sensitivity, which is vital for sensing dangerous materials.



[3]

Figure 2: Illustration of the emission difference with isolated and wired PPE.

In studies in which sugar-substituted PPEs were quenched with mercury and lead ions, Kim has observed a phenomenon known as cooperative quenching. The experiments concluded that two or more sugar substituents could participate in the complexation of a single mercury ion, which would give significantly higher Ksv values than a PPE not experiencing cooperative quenching. [4]

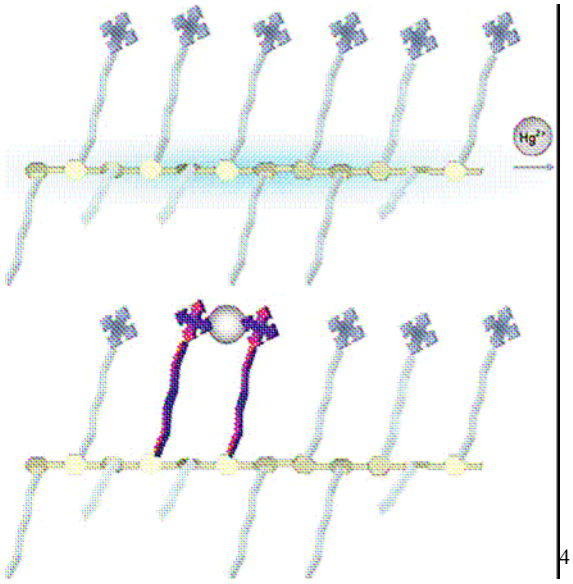


Figure 3: Cooperative quenching is illustrated as two sugar-PPE sensors bind to mercury

Un-aggregated PPEs in solution exhibit a short fluorescence lifetime of approximately .3 to .5 ns.[3] These fluorescence lifetimes do change upon the addition of quencher. As a result, static quenching is the established quenching mechanism in PPE fluorescence quenching. In these cases of static quenching, the Stern-Volmer constant is equal to the binding constant of the quencher to the fluorophore (PPE).

Schanze reports anionic PPEs that are significantly quenched by methyl viologen. In addition to demonstrating anionic PPE quenching, this study also reflects the differences of quenching when immersed into different solvents. In methanol, sulfonated PPE and its analog expressed Ksv values of 1.4×10^{7} and 2.2×10^{4} M^{-1}, respectively. In water, the Ksv values were 2.7×10^{7} M^{-1} and 7.0×10^{3} M^{-1}, respectively. This polymer was more aggregated in water but not in methanol because of the polymer's more polar character. This aggregation may result in an increased self-quenching.

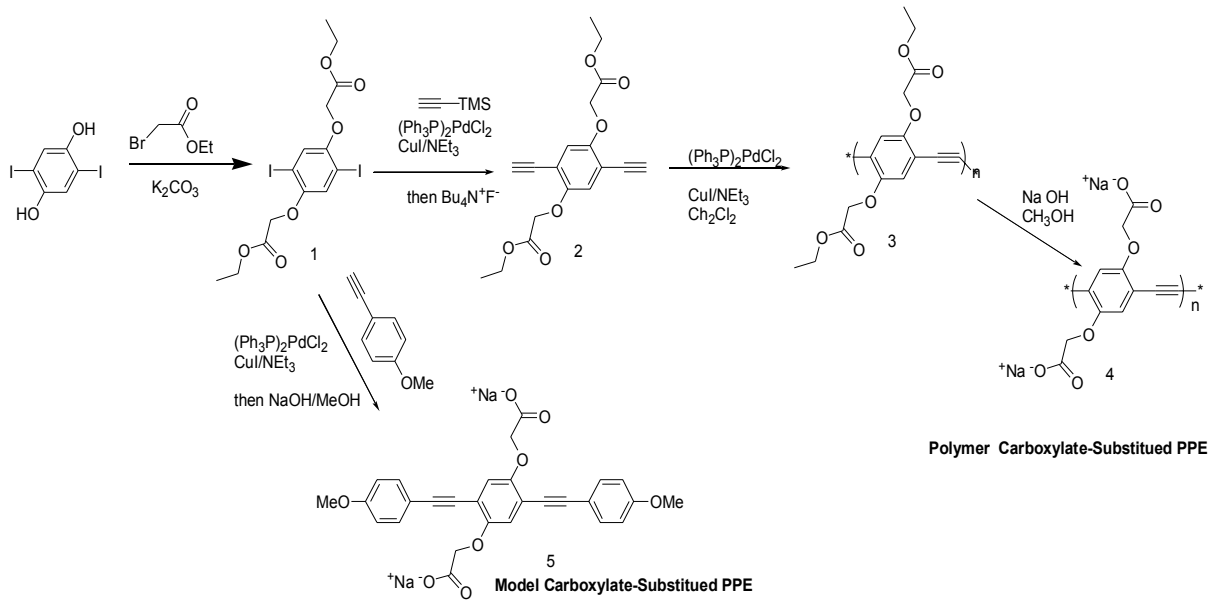
Quenching studies have focused primarily on the mechanism of PPE quenching as well as the effects of aggregation in chemical sensing.

Procedure & Methods

The methods for re-evaluating fluorophore concentration dependence on Stern-Volmer quenching involves synthesizing un-aggregatated PPEs, preparing stock and sample solutions, and conducting quenching experiments.

Synthesis of PPE

Before any quenching studies can take place, the polymers must first be synthesized. One of the polymers used for the concentration-dependent quenching studies was also used in the sensing of lead ions. As indicated in **scheme 1**, synthesis of the polymer takes place by starting from 2,5 diiodohydroquinone in the presence of potassium carbonate, which yields compound 1. Trimethylsilylacetylene and a $Pd(PPh_3)_2Cl_2$ / CuI catalyst are placed with triethylamine to produce compound 2. Coupling of 1 and 2 at 50 $^{\circ}$C for 72 hours produced polymer 3 (carboxylate- substituted PPE) with a 93% yield and molecular weight of 12 x 10^3 g/mole. A solution of polymer 3 in methanol was treated with NaOH to yield the water-soluble carboxylate-substituted PPE.



Scheme 1: Synthetic route for the synthesis of water-soluble carboxylated PPEs

Preparation of Stock and Sample Solutions:

After obtaining synthesized PPE, a stock solution of concentration 5 x 10^{-4} M was prepared by dissolving the appropriate amount of PPE into 20 mL of deionized water. The stock solution would serve as the principal source of PPE for the preparation of the varying concentrations of PPE sample solutions. Using a pre-determined volume of the PPE stock solution, a series of sample solutions were prepared via serial dilution as seen below in **figure 4**.

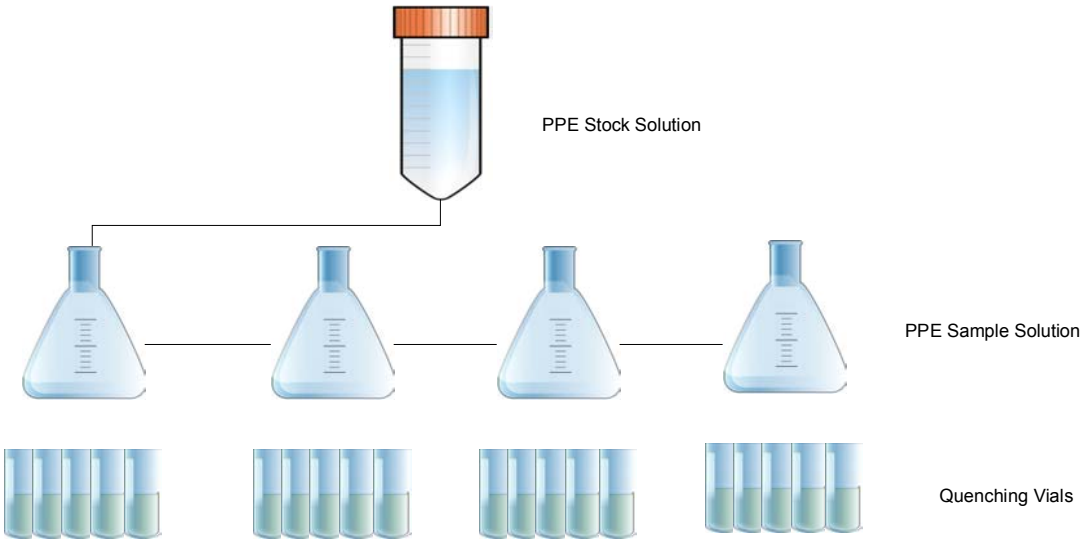


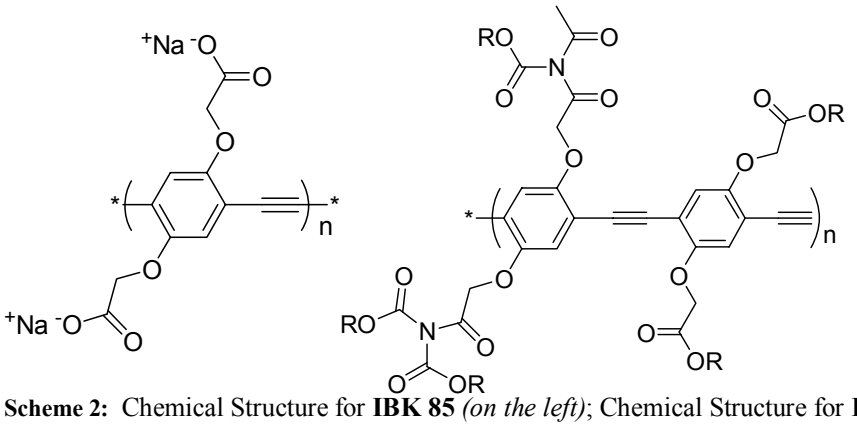
Figure 4: A concentrated PPE stock solution is prepared and used to make up all diluted PPE samples. Schematic also indicates that quenching experiments take place for each PPE sample solution.

Vial #	Volume of Sample PPE solution (mL)	Volume of $TbCl_3$ quencher (µL)
0	5	0
1	5	10
2	5	20
3	5	30
4	5	40
5	5	50
6	5	60
7	5	70
8	5	80
9	5	90
10	5	100

Table 1: The fluorescence quenching of each vial is measured using fluorimetry and a quenching curve is generated for each vial. The concentration of quencher added depends on PPE sensitivity.

Quenching Experiments

For these quenching experiments, $TbCl_3$ was used as the quencher. As described earlier, PPE substituents can affect the sensitivity to a particular quencher so a method of guess and check was applied to determine the appropriate quencher concentration range for the experiments. Before data collection occurs, a volume of 10 μL of $TbCl_3$ of either .01M, .001 M or .0001 M was added to three different vials containing 5mL of PPE solution. The different quenching effects were observed and an appropriate range was determined. After determining the appropriate quencher concentration range, a quenching experiment was conducted for each concentration of PPE sample solution. A series of 11 vials were prepared that all contained the same volume and concentration of PPE, but also contained different volumes of $TbCl_3$. The contents of each vial were transferred into a cuvette and measured by spectrofluorimetry. PPEs typically absorb at 430 nm, and an emission spectra was obtained for each vial with excitation at 405 nm. The quenching effects were examined for each concentration of PPE so that the differences in Stern-Volmer activity could be observed. Both PPEs identified as **IBK 85** and **IBK 146** were used for the quenching experiments.



Scheme 2: Chemical Structure for **IBK 85** *(on the left)*; Chemical Structure for **IBK 146** *(on the right)*

Results

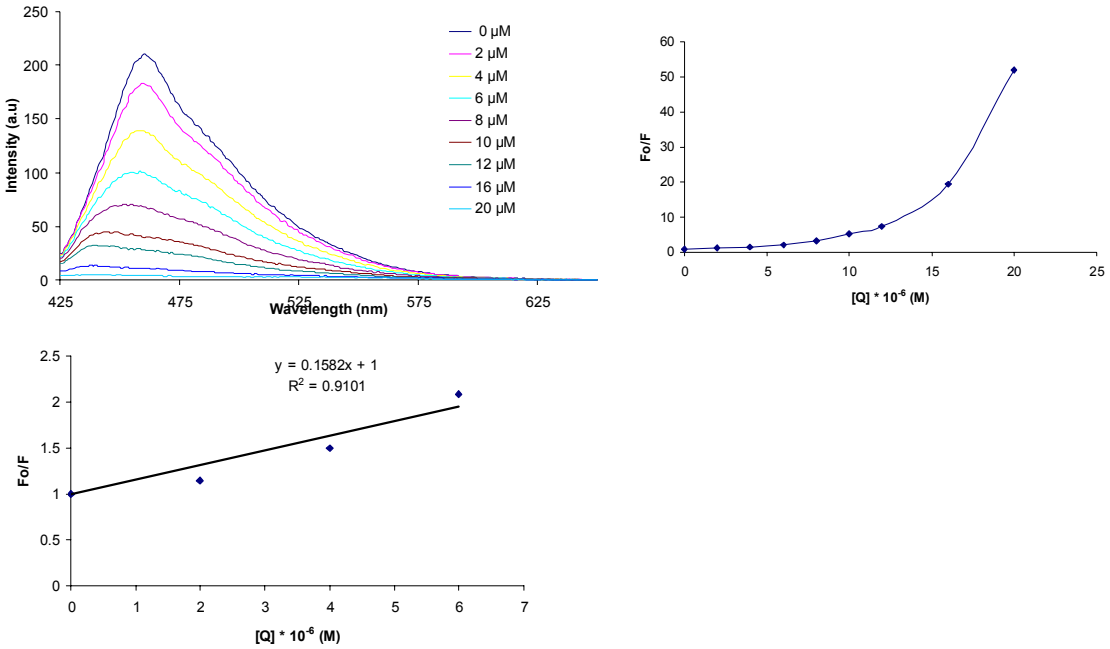


Figure 5: The quenching curves, and Stern-Volmer plots for PPE **IBK 85** at 5 x 10^{-5} M

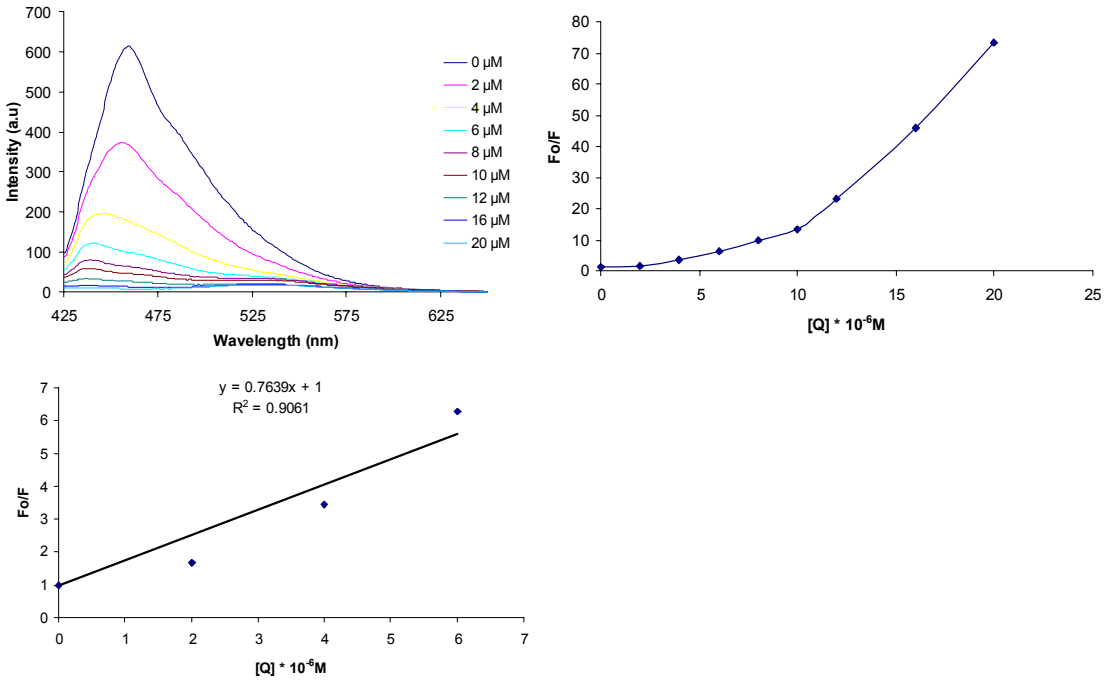


Figure 6: The quenching curves, and Stern-Volmer plots for PPE **IBK 85** at 1 x 10^{-5} M

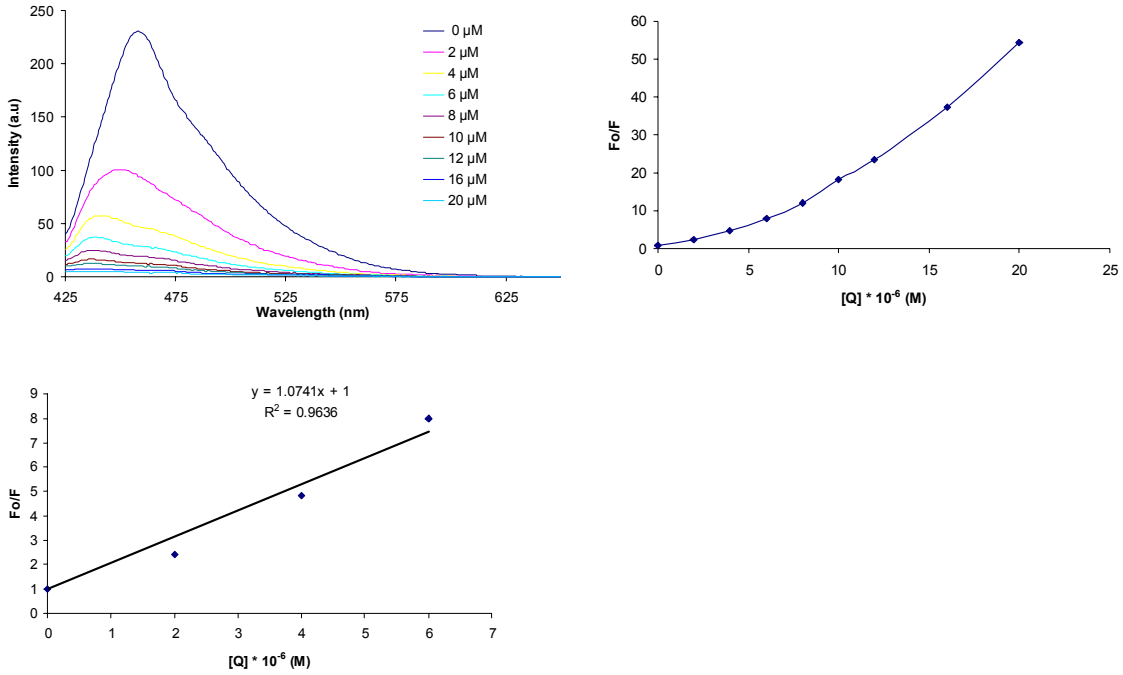


Figure 7: The quenching curves, and Stern-Volmer plots for PPE **IBK 85** at 5 x 10^{-6} M

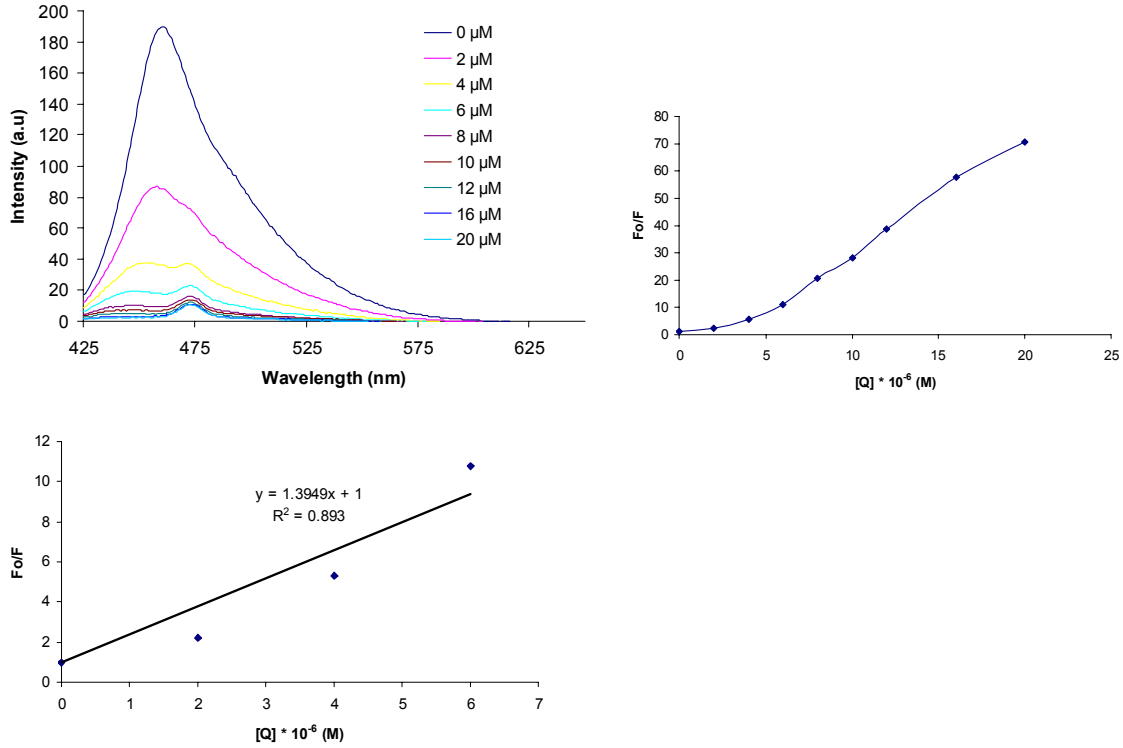


Figure 8: The quenching curves, and Stern-Volmer plots for PPE **IBK 85** at 1 x 10^{-6} M

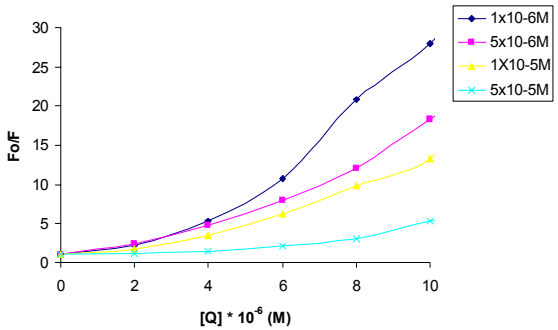


Figure 9: Summary of Stern-Volmer plots for PPE **IBK 85**

PPE Concentration (M)	**K_{sv} (M^{-1})**	**Linear Regression Correlation Coefficient**
5×10^{-5}	1.6×10^{5}	.91
1×10^{-5}	7.6×10^{5}	.91
5×10^{-6}	1.0×10^{6}	.96
1×10^{-6}	1.4×10^{6}	.89

Table 2: Stern-Volmer constants in quenching curve are generated for each vial. The concentration of quencher added depends on PPE sensitivity.

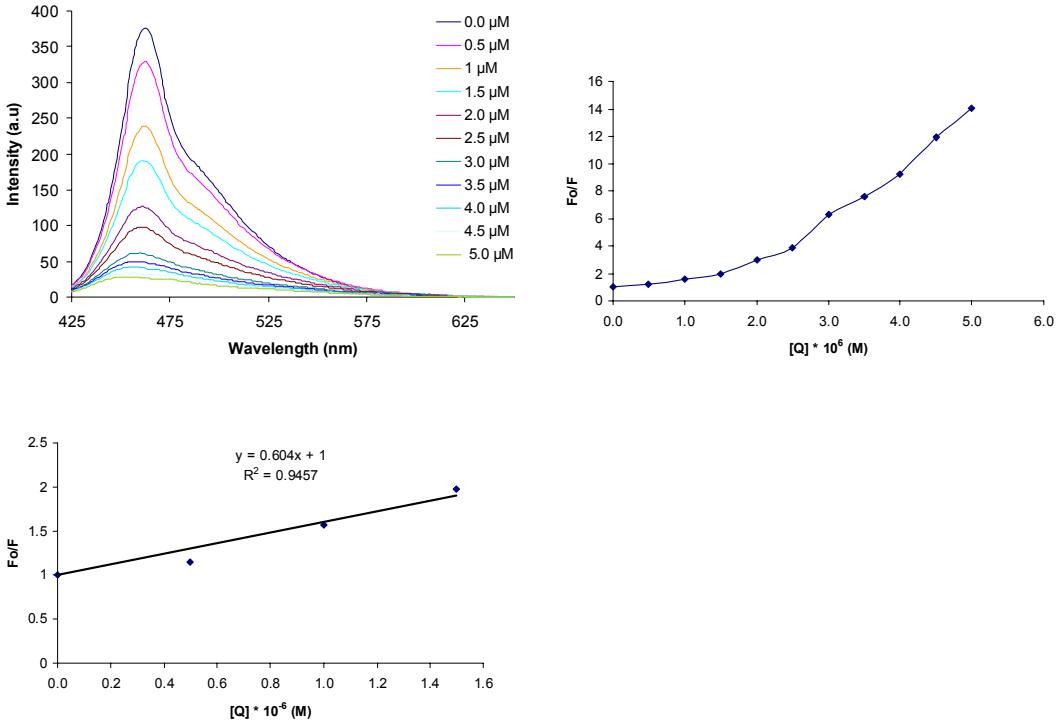


Figure 10: The quenching curves, and Stern-Volmer plots for PPE **IBK 146** at 5 x 10^{-6} M

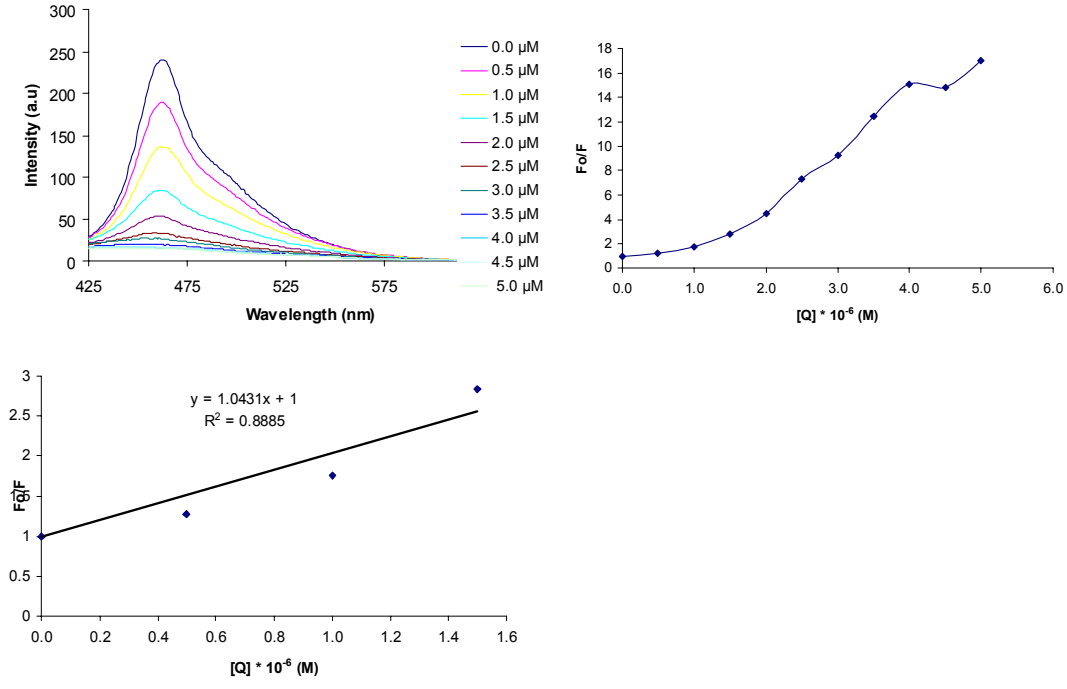


Figure 11: The quenching curves, and Stern-Volmer plots for PPE **IBK 146** at 1 x 10^{-6} M

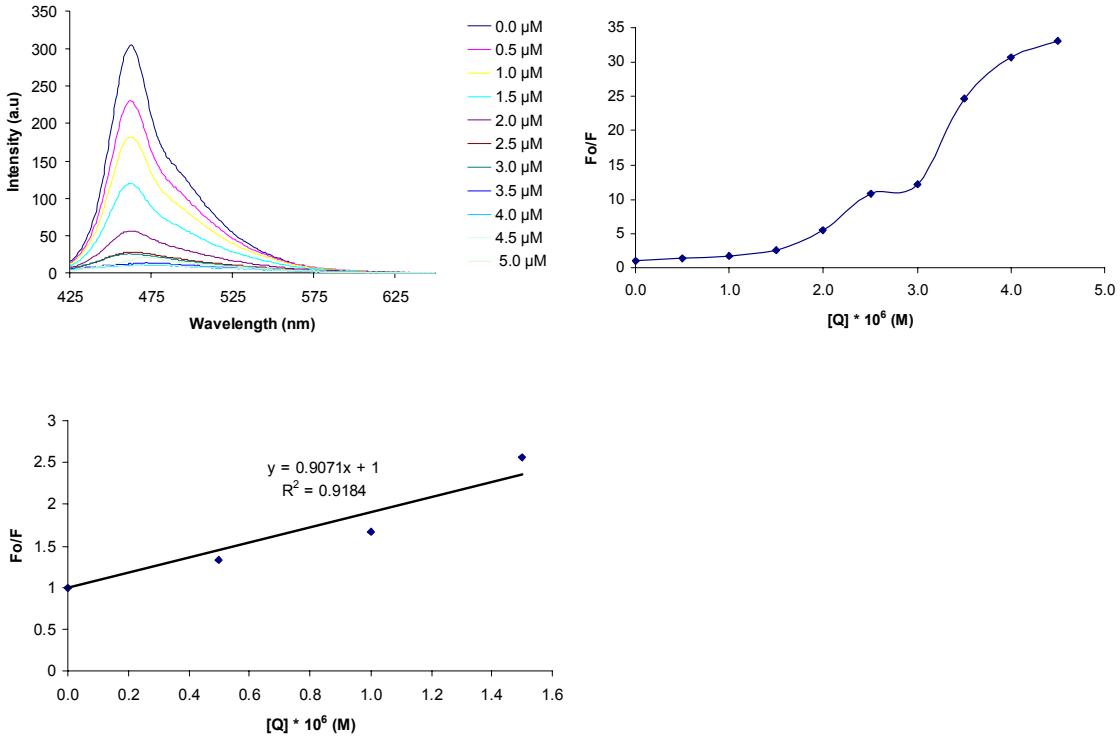


Figure 12: The quenching curves, and Stern-Volmer plots for PPE **IBK 146** at 5 x 10^{-7} M

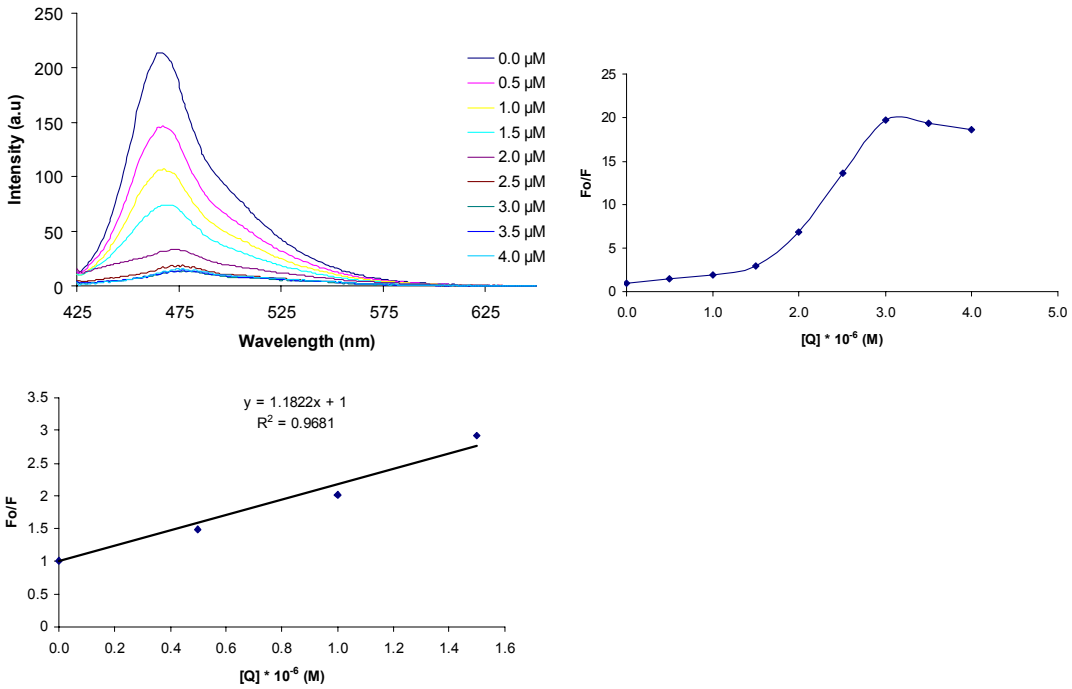


Figure 13: The quenching curves, and Stern-Volmer plots for PPE **IBK 146** at 1 x 10^{-7} M

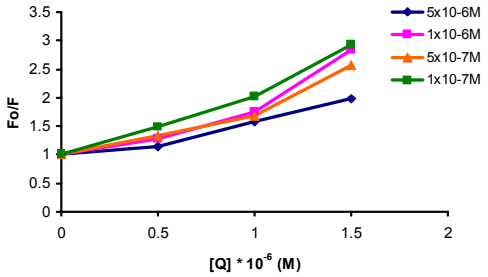


Figure 14: Summary of Stern-Volmer plots for PPE **IBK 146**

PPE Concentration (M)	**K_{sv} (M^{-1})**	**Linear Regression Correlation Coefficient**
5×10^{-6}	6.0×10^{5}	.95
1×10^{-6}	1.0×10^{6}	.89
5×10^{-7}	9.1×10^{5}	.92
1×10^{-7}	1.2×10^{6}	.89

Table 3: Summary of Stern Volmer constants for PPE **IBK 146**

Discussion

Data was obtained for PPE **IBK 85** quenching with terbium (III) chloride at PPE concentrations of 5 x 10^{-5} M, 1 x 10^{-5} M, 5 x 10^{-6} M, and 1 x 10^{-6} M. An appropriate quenching range was determined to be between 0 and 20 μM of $TbCl_3$ through the procedure described in the methods section. Quenching experiments were conducted for each concentration at the specified quenching range, and the fluorescence of each solution containing quencher was measured. The maximum intensity for the solutions without quencher was seen at 461nm, and as a result, the intensities at this wavelength were compared to intensity without quencher. This procedure gave the ratio of $F_o/F_{[Q]}$, which was plotted against the quencher concentration range. A fluorescence spectrum, quenching curve, and Stern-Volmer plot are present for each concentration quenching experiment in figures **5 -8**. The quenching curve demonstrates the quenching behavior over the entire pre-determined quenching range, while the other plot zooms in on the more classical linear Stern-Volmer activity.

As seen in figure **5**, the quenching experiment of concentration of 5 x 10^{-5} M PPE **IBK 85** had an apparent Ksv constant of 1.6 x 10^5 M^{-1}. Figure **6** demonstrates an apparent Ksv constant of 7.6 x 10^5 M^{-1} at a PPE **IBK 85** concentration of 1 x 10^{-5} M. PPE **IBK 85 of** concentration 5 x 10^{-6} M yielded an apparent Ksv constant 1.0 x 10^6 M^{-1}, as seen in figure**7**. Figure **8** illustrates that an apparent Ksv constant of 1.4 x 10^6 M^{-1} was observed for PPE **IBK 85** concentration of 1 x 10^{-6} M.

Figure **9** shows the quenching curves for all the experimented PPE concentration are one plot, and demonstrates that sensitivity for the terbium quencher increases with decreasing PPE concentration. PPE sensitivity to the terbium quencher was at its lowest

at 5 x 10^{-5} M and sensitivity was at its highest at the concentration of 1 x 10^{-6} M. As seen in table **2**, all the linear regression coefficients were approximately .9, which suggests a high linear correlation. This high linear correlation between the quencher concentration and $F_o/F_{[Q]}$ is in accordance with the Stern-Volmer equation **1**.

The results suggest there is a fluorophore concentration effect on Stern-Volmer quenching. Generally, there is a higher sensitivity observed with lower concentration of PPE than with higher concentrations. More dilute concentrations of PPE have high Stern-Volmer constant in quenching with terbium (III) chloride. As figure **5- 8** demonstrate, concentrations varying from 5 x 10^{-5} M to 1 x 10^{-7} M had increasing Stern-Volmer constants, which suggest that there is a greater sensitivity to the terbium quencher with decreased PPE concentration.

Though this data agrees with the prediction of increased sensitivity with decreased PPE concentration, this data comes with a cautionary note because there are some problems with the fluorescence spectrum for each of the PPE **IBK 85** concentrations. Blue shift is a phenomenon by which the maximum emission of the PPE shifts towards the blue region of the electromagnetic spectrum. The major suggestion for this phenomenon among this data set is that longer chains of PPE are efficiently quenched first, and that unquenched shorter chains are attributing to a lower maximum emission wavelength. This phenomenon is similar to Swager's work in comparing the molecular wire chemo sensor to an isolated chemo sensor, where longer chains of PPE are completely quenched before the shorter chains. Despite this cautionary note, it is important to acknowledge that the Stern-Volmer constants changed with fluorophore PPE concentration and seem in accordance with predicted behavior.

Since blueshift activity was observed in the PPE **IBK 85** solutions, quenching studies were conducted with another PPE. As seen below in figure **15**, PPE **IBK 146** has a lesser degree of blueshift than PPE **IBK 85**.

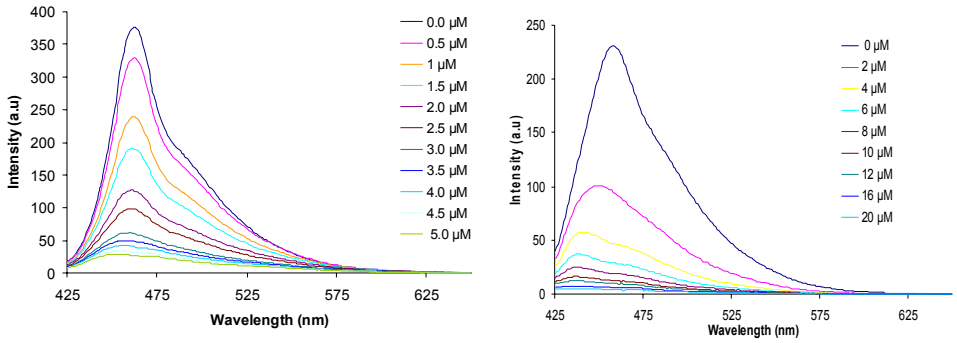


Figure 15: *On the left* PPE **IBK 146** at 5x 10^{-6} M does not have any blueshift activity
On the right PPE **IBK 85** shows blueshift activity at 5x 10^{-6} M

Data was obtained for PPE **IBK 146** quenching with terbium (III) chloride at PPE concentrations of 5 x 10^{-6} M, 1 x 10^{-6} M, 5 x 10^{-7} M, and 1 x 10^{-7} M. An appropriate quenching range was determined to be between 0 and 5 µM of $TbCl_3$ through the procedure described in the methods section. Quenching experiments were conducted for each concentration at the specified quenching range, and the fluorescence of each solution containing quencher was measured using the same procedure for PPE **IBK 85**. Using the same technique to obtain Stern-Volmer plot for **IBK 85**, the ratio of $F_o/F_{[Q]}$ was plotted against the quencher concentration range. A fluorescence spectrum, quenching curve, and Stern-Volmer plot are present for each concentration quenching experiment in figures **10-13**.

As figure **10** illustrates, the quenching experiment for the concentration of

5×10^{-6} M PPE **IBK 146** had an apparent Ksv constant of $6.0 \times 10^{5}\ M^{-1}$. Figure **11** shows an apparent Ksv constant of $1.0 \times 10^{6}\ M^{-1}$ at a PPE **IBK 146** concentration of 1×10^{-6} M. PPE **IBK 146** of concentration 5×10^{-7} M yielded an apparent Ksv constant $9.1 \times 10^{5}\ M^{-1}$, as seen in figure **12**. Figure **13** points to an apparent Ksv constant of $1.2 \times 10^{6}\ M^{-1}$ was observed for PPE **IBK 146** concentration of 1×10^{-7} M.

When considering the elimination of blue shift activity as seen in PPE **IBK 146**, the data agrees with the prediction of increased sensitivity with decreased PPE concentration. As figure **14** indicates, PPE sensitivity was minimized at concentration of 5×10^{-6} M and maximized at 1×10^{-7} M. Though the exact dependence of fluorophore cannot be equated to a variable in the Stern-Volmer equation, the data leads to the conclusion that there is some dependence on the fluorophore concentration in Stern-Volmer behavior. This research has grave implications on chemo sensing and suggests that lesser concentrated chemo sensors will provide greater sensitivity. Future research needs to involve different PPEs with different substituents to allow for greater insight in the general fluorophore concentration effect of Stern-Volmer quenching on conjugated polymers.

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